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Molecular Weight of Some Human and Cow Caseins

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The molecular weights of a number of caseins isolated from human and cow milks have been determined by sedimentation equilibrium. The predominant human caseins, which differ only in their content of phosphorus, have a molecular weight of approximately 25,000 daltons, a value similar to that of cow β- and γ-caseins. Other cow caseins (R, S, and TS) have molecular weights of 13,000–16,000. All values obtained by these measurements agree with minimum molecular weights calculated from content of tryptophan assuming the presence of one residue per molecule.

Monomer molecular weights of 25,000–28,000 daltons have been obtained by physical measurements on the isolated bovine casein components, β- and α_{s1}-caseins (1–5). Spies (6) has determined the tryptophan content of these proteins, and minimum values, based on 1 and 2 moles of tryptophan per mole of β- and α_{s1}-casein, respectively, are consistent with the above molecular weights. Several other caseins have recently been separated from whole bovine casein and a number of human casein components, which have been identified by their electrophoretically separable bands, have also been individually isolated (7). Very little information is available on the comparison of human milk proteins with those of cow's milk, either from the standpoint of composition or molecular size. In order to better our understanding of similarities and differences of the casein systems, the molecular weight of human caseins and some minor caseins from cow milk were measured. The ultracentrifugally determined molecular weights of these proteins are in good agreement with their minimum molecular weights calculated from the content of tryptophan.

The major portion of acid-precipitated human casein consists of six components identifiable by disc-gel electrophoresis (pH 9.6, 4 M urea) and tentatively named caseins I–VI in order of their decreasing mobility (7). Caseins I–VI were isolated by DEAE–cellulose chromatography of human casein and were found to contain the same numbers of all amino acid residues, and to differ solely in their phosphorus content. Casein I shows the fastest mobility and contains 5 atoms of phosphorus, presumably present as phosphate groups in phosphorylated hydroxyamino acids; the other caseins decrease stepwise in mobility and phosphorus content to the slowest, casein VI, which is free of phosphorus. Molecular weight determinations were made on caseins I, IV, and VI, which contain 5, 2, and 0 atoms of phosphorus, respectively, per molecule of 25,000 daltons.

Bovine γ-casein and other caseins, TS-A, TS-B, R-, and S-caseins,[1] have recently been isolated from milks of individual cows by chromatography of acid-precipitated casein (8–10). The γ-caseins, depending on the source, occur as electrophoretically distinct polymorphs designated A^1, A^2, A^3, and B.

The TS-A, TS-B, R-, and S-caseins are obtained in a fraction eluted prior to the γ-caseins when casein is chromatographed on a DEAE-cellulose column. The fraction

[1] TS-(temperature-sensitive) casein and the S-casein, likewise, are insoluble at pH 8 and 25° but dissolve at 3°; R-casein is soluble at both temperatures.

eluting with the starting buffer of pH 8.3, 0.005 M phosphate, is designated the TS-fraction. In this fraction is found either the R-casein and TS-A pair or the S-casein and TS-B pair, depending on whether the original protein is typed A or B with respect to γ-casein. Isolation of these proteins was accomplished by rechromatography of the appropriate fractions. The elution sequence was either TS-A, then R-casein or S-casein, followed by TS-B. The details of this fractionation will be described elsewhere.

Human caseins and the above bovine caseins were analyzed for tryptophan by procedure U of Spies (6). The percentages found are shown in Table I. Our value of 0.78% for γ-casein A^1 is slightly lower than the tryptophan content, 0.82%, for γ-casein A^2 and B determined for us by Spies (8). A value for γ-casein A^3 is not included because of difficulties encountered in its analysis.

Ultracentrifugal measurements of molecular weight were made by the high-speed equilibrium method of Yphantis (11). The Rayleigh interference optics of the ultracentrifuge were aligned with all the components exactly on the optical axis (12) and the camera lens was focused on the ⅔ plane of the sapphire-windowed cell. A liquid column height of 3 mm was used routinely. Rotor speeds selected were 33,450 rpm for the human, γ, and S-caseins, and 42,040 rpm for the TS and R-caseins. At these speeds, the upper portion of the liquid columns were free from gradients at the highest concentrations used, approximately 0.7 mg/ml. These speeds and concentrations, although determined empirically, are not far from the optimum calculable by the method of Teller *et al.* (13). All runs were made at 4.0°, with the exception of TS-casein, which was run at 2.0°. At these temperatures it was found necessary to fill the ultracentrifuge cell in a special way. The cell was first tightened to 70 in-lb and chilled in the refrigerator to about 4°. The cell was then rapidly filled with cold solution, tightened to 110 in-lb, and placed in the pre-chilled rotor. Tightening the cell fully before chilling led to distortion of the sapphire windows, as was evidenced by severe blurring of the fringes.

TABLE I

TRYPTOPHAN CONTENT OF CASEINS

Casein type	% Tryptophan
Human casein	
Casein I	0.71
Casein IV	0.73 ± 0.01[a]
Casein VI	0.77
Cow casein	
γ-Casein A^1	0.78
γ-Casein A^2	0.82[b]
γ-Casein B	0.82[b]
R-Casein	1.49
S-Casein	1.24
TS-Casein A	1.31 ± 0.01[c]
TS-Casein B	1.52

[a] Average of four determinations.

[b] Measurements of J. R. Spies, reported in Ref. (8).

[c] Average of two determinations.

Solutions of γ-casein A^3, TS-casein B, and S-casein were prepared for ultracentrifugation by dissolving the caseins in 0.01 M imidazole (free base) pH 8.0, at a concentration of 0.6–0.7 mg/ml. Solution was effected by stirring the mixture at 4° and, if necessary, by adding a small drop of dilute ammonium hydroxide, after which the solutions were dialyzed at least 4 hr at 4° against a large excess of pH 8, 0.01 M imidazole solution. This procedure was satisfactory for the three caseins mentioned above and the plots of logarithm of fringe displacement vs r^2 gave straight lines. The molecular weights were determined from the slopes. For R-casein and the human caseins, however, the equilibrium plot showed a severe downward curvature toward the bottom of the cell, indicating nonideal behavior (14, 15). Consequently, the R-casein and caseins I, IV, and VI were remeasured in 0.01 M NaCl adjusted to pH 10 with NaOH. Experiments in this solvent gave completely straight lines throughout the liquid column when ln of fringe shift was plotted against r^2. γ-Casein A^3 and TS-casein B were also remeasured at concentrations of about 0.7, 0.5, and 0.2 mg/ml in 0.02 M NaCl, pH 10.6, using the six-channel interference cell of Yphantis (11). Essentially identical molecular weights, independent of concentration, were found under these conditions. Their

average is shown in Table II. The molecular weight of γ-casein A^1 was also measured over a similar 3-fold concentration range in the above 0.02 M NaCl solvent. Based on the weight of the lyophilized sample, uncorrected for moisture, the concentrations were 0.74, 0.44, and 0.25 mg/ml. The plot of ln of fringe shift vs r^2 was in each case straight throughout the liquid column; however, the apparent molecular weights obtained increased significantly with decreasing concentration, and extrapolate linearly to 24,700 daltons at zero concentration. This value is given in Table II. Such a trend was not seen in either γ-casein A^3 or TS-casein B. This concentration dependence, coupled with lack of curvature in the plot, might indicate nonideal behavior in this solvent together with some residual heterogeneity (11).

Electrostatic effects may also be contributing to the apparent molecular weights obtained. Due to the strong tendency of the caseins to be aggregated by salts, the ionic strength of the medium was deliberately kept as low as possible, and charge effects are not surprising. The negative concentration dependence of the molecular weight of γ-casein A^1 is in the direction that such effects would cause. The absence of any trend for the TS-casein B and γ-casein A^3, on the other hand, indicates that at least in these latter proteins, the virial coefficients are not exceptionally large. Remeasurement in strongly dissociating solvents, such as 6 M guanidine HCl is indicated. However, lack of more sample at this time precluded repetition.

Partial specific volumes ($\bar{V}$) were calculated, using the method of McMeekin *et al.* (16), for the caseins for which total amino acid composition data were available. These are included in Table II; in the cases in which a calculated value was unavailable, 0.75 ml/g was assumed. The partial specific volumes, 0.750 and 0.751 ml/g, calculated from the compositions of γ-casein A^1 and A^3 are in good agreement with the value 0.750 ml/g obtained by physical measurements on an early preparation of γ-casein (17).

The agreement between the minimum molecular weight based on one tryptophan residue and the ultracentrifugally determined molecular weight is seen in Table II. These data show that these rare caseins, like the more common α_{s1}- and β-caseins, are dissociated into their monomeric units under conditions of low ionic strength, high pH, and low temperature. Furthermore, the occurrence of a single tryptophan residue per molecule in these caseins appears to be a unique property of a majority of the caseins in human and cow milks.

The human caseins I, IV, and VI are similar to bovine α_{s1}-, β-, and γ-caseins in molecular weight. As might be expected, these three human caseins, which differ

TABLE II

MOLECULAR WEIGHT OF CASEINS

Casein type	$\bar{V}$	Solvent	Mol wt ultracentrifuge	Min mol wt based on one tryptophan
Casein I	0.751	0.01 M NaCl–NaOH, pH 10	25,750	28,800
Casein IV	0.755	0.01 M NaCl–NaOH, pH 10	25,550	27,900
Casein VI	0.758	0.01 M NaCl–NaOH, pH 10	25,450	26,500
γ-Casein A^1	0.750	0.02 M NaCl–NaOH, pH 10.6	24,700[a]	26,200
γ-Casein A^3	0.751	0.01 M imidazole, pH 8	21,700	
		0.02 M NaCl–NaOH, pH 10.6	22,800 ± 400[b]	
R-Casein	0.75[c]	0.01 M NaCl–NaOH, pH 10	13,250	13,700
S-Casein	0.75[c]	0.01 M imidazole, pH 8	16,150	16,500
TS-Casein B	0.75[c]	0.01 M imidazole, pH 8	12,850	13,400
		0.02 M NaCl–NaOH, pH 10.6	12,900 ± 400[b]	

only in their phosphorus content, show no significant differences in molecular weight.

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